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Acute Subcutaneous Toxicity of Physostigmine Salicylate in Sprague-Dawley Rats

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MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

November 1988

Toxicology Series: 214

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

Acute Dermal Toxicity of Diethyleneglycol Dinitrate in Rabbits (Toxicology Series 155)--
Brown and Korte

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The acute subcutaneous toxicity of physostigmine salicylate was determined in male and female Sprague-Dawley rats using the single-dose method. The median lethal dose was 1.78 mg/kg ± 0.07 mg/kg for male and 1.54 mg/kg ± 0.08 mg/kg for female rats. Clinical signs observed were primarily related to changes in behavior, such as tremors, hypertonia, irritability, somnolence, inactivity, and ataxia. Other noted clinical signs included lacrimation, salivation, and diarrhea. The duration of the clinical signs was acute. Most animals were exhibiting signs by 1 hour after dosing and had either died or returned to normal by 4 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place physostigmine salicylate in the highly toxic class.

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ABSTRACT

The acute subcutaneous toxicity of physostigmine salicylate was determined in male and female Sprague-Dawley rats using the single-dose method. The median lethal dose was 1.78 mg/kg ± 0.07 mg/kg for male and 1.54 mg/kg ± 0.08 mg/kg for female rats. Clinical signs observed were primarily related to changes in behavior, such as tremors, hypertonia, irritability, somnolence, inactivity, and ataxia. Other noted clinical signs included lacrimation, salivation, and diarrhea. The duration of the clinical signs was acute. Most animals were exhibiting signs by 1 hour after dosing and had either died or returned to normal by 4 hours after dosing. According to the classification scheme of Hodge and Sterner, these results place physostigmine salicylate in the highly toxic class.

Key Words: Acute Subcutaneous Toxicity, Physostigmine Salicylate, Rat, RA V

PREFACE

TYPE REPORT: Acute Subcutaneous Toxicity GLP Study Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5425
Project Cfficer: LTC J. von Bredow, PhD, MSC

PROJECT/WORK UNIT/APC: 3M162734A875/308/TLE0

GLP STUDY NUMBER: 87005

STUDY DIRECTOR: Don W. Korte, Jr., PhD, MAJ, MS

PRINCIPAL INVESTIGATOR: Denzil F. Frost, MS, DVM, CPT, VC

PATHOLOGIST: Charles B. Clifford, DVM, PhD, MAJ, VC
Diplomate, American College of Veterinary Pathologists

DATA MANAGER: Yvonne C. LeTellier, BS

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, retired SOPs, raw data, analytical, stability, and purity data of the test compound, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Physostigmine Salicylate

INCLUSIVE STUDY DATES: 21 May - 18 June 1987

OBJECTIVE: The objective of this study was to determine the acute subcutaneous toxicity of physostigmine salicylate in male and female Sprague-Dawley rats.

ACKNOWLEDGMENTS

SP4 Dean K. Magnuson, BS, SP4 Joel B. Seewald, BS, SGT Charles J. Freedman, and SGT Tammie R. Heineman provided research assistance and animal care; Conrad R. Wheeler, PhD, and SGT John R. G. Ryabik, BS, provided chemical preparation and analysis; Michael Pearce provided data assimilation assistance; Rick Katona and Obie Goodrich provided animal care and facility management; and Marie Rogers provided secretarial assistance.

SIGNATURES OF PRINCIPAL SCIENTISTS AND MANAGERS INVOLVED IN THE STUDY

We, the undersigned, declare that study number 87005 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.

Don W. Korte 27 Oct 88
DON W. KORTE, JR., PhD/DATE
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DEPARTMENT OF THE ARMY

LETTERMAN ARMY INSTITUTE OF RESEARCH
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REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 28 October 1988

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 87005

1. This is to certify that in relation to LAIR GLP Study 87005, the following inspections were made:

06 April 1987	-	Protocol Review
03 June 1987	-	Dosing
03 June 1987	-	Observations - Phase I
17 June 1987	-	Final Sacrifice
18 June 1987	-	Final Observations - Phase II
04 November 1987	-	Scoring Slides, Micronuclei Assay

2. The institute report entitled "Acute Subcutaneous Toxicity of Physostigmine Salicylate in Sprague-Dawley Rats," Toxicology Series 214, was audited on 22 December 1987. and the Pathology Report audited on 2 February 1988.

Carolyn M. Lewis

CAROLYN M. LEWIS
Chief, Quality Assurance

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Acute Subcutaneous Toxicity of Physostigmine Salicylate in Sprague-Dawley Rats -- Frost and Korte

INTRODUCTION

Soman, the primary nerve agent utilized by threat forces, is refractory to the standard antidotal therapy, atropine and pralidoxime (2-PAM), fielded by the US Army. Consequently, the highest priority has been placed on fielding a more effective treatment regimen. A regimen incorporating pyridostigmine as a prophylactic agent, combined with standard atropine/2-PAM therapy, has proven extremely effective in reducing mortality of Rhesus monkeys to multilethal concentrations of soman (1). However, these animals require a prolonged period of recovery during which they are completely incapacitated. This has been attributed to the quaternary nature of pyridostigmine, which does not cross the blood-brain barrier and thus only protects the peripheral nervous system. Consequently, a tertiary carbamate, physostigmine, has been proposed for the prophylactic regimen since it would protect the central nervous system in addition to the peripheral nervous system. Experimental studies support this hypothesis as animals pretreated with physostigmine before exposure to soman recover at a faster rate than animals pretreated with pyridostigmine (2,3). An enhanced rate of recovery of soldiers from a multilethal exposure to soman would produce a decided advantage in maintaining a fully functional military unit during a future conflict.

The only approved formulation of physostigmine is for intravenous administration, which is not a feasible option for the proposed prophylactic therapy. Either the oral or dermal route of administration for prophylactic therapy would be feasible. However, even though physostigmine has been available for more than a century (4), little directed research on its toxicology following oral or dermal administration has been conducted. Consequently, the Division of Toxicology, Letterman Army Institute of Research, was tasked by the US Army Medical Research Institute of Chemical Defense to provide an acute and subchronic toxicity profile of physostigmine salicylate following oral and subcutaneous administration.

Objective of Study

The objective of this study was to determine the acute subcutaneous toxicity of physostigmine salicylate in male and female Sprague-Dawley rats.

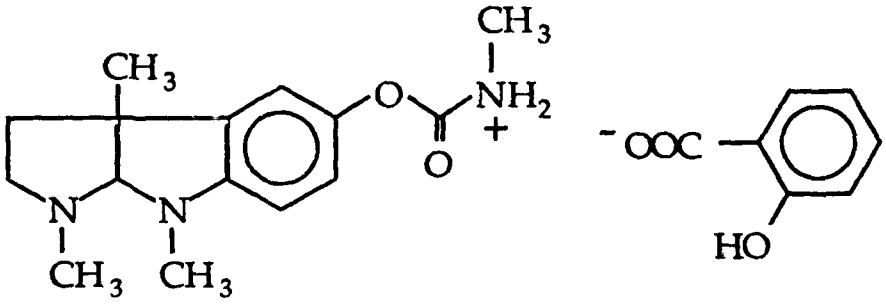
MATERIALS

Test Substance

Chemical Name: Physostigmine salicylate

Chemical Abstracts Service Registry No.: 57-64-7

Chemical Structure:



Molecular Formula: $C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$

Source: Mr. William Ellis
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Requested by LTC J. von Bredow, USAMRICD

Other test substance information is presented in Appendix A.

Vehicle

The vehicle for physostigmine salicylate was sterile water (Abbott Labs, North Chicago, IL 60064). The expiration date was 1 February 1989, and the lot number was 01-075-FW.

Animal Data

Fifty-seven male and 57 female Sprague-Dawley rats (Bantin-Kingman, Inc., Fremont, CA) were used for this study. They were identified individually with tail tattoos. Two males and two females were randomly selected for quality

control necropsy. The animal weights on receipt (21 May 87) ranged from 117 to 171 g. Additional animal data appear in Appendix B.

Husbandry

Rats were caged individually in stainless steel wire-mesh cages with automatic flushing dumptanks. No bedding was used in any of the cages. The diet, fed *ad libitum*, consisted of Certified Purina Rodent Chow® Diet 5002 (Ralston Purina Company, St. Louis, MO); water was provided by continuous drip from a central line. The animal room temperature was maintained in a range from 19.2°C to 24.8°C with a relative humidity range of 42% to 63% with occasional spikes to as high as 87% during room cleaning. The photoperiod was 12 hours of light per day.

METHODS

Group Assignment/Acclimation

Study rats were initially randomized into 5 dose groups of 10 males and 10 females each, and vehicle control groups of 5 males and 5 females each. Allocation was accomplished using a computer-based, stratified, weight-biased method. The Beckman TOXSYS® Animal Allocation Program was used in conjunction with a Beckman TOXSYS® Data Collection Terminal. The animals were acclimated for 13 days before the day of dosing. During this period they were observed daily for signs of illness.

Dosage Levels

The results of the ALD determination were that the median lethal dose (MLD) was between 1 and 2 mg(base) per kg. Based on these data, test dosages were selected (Table 1).

Compound Preparation

A stock solution of physostigmine salicylate was prepared in sterile water for injection. Specific concentrations for dosing were then prepared by diluting aliquots of the stock solution with sterile water for injection.

TABLE 1: Physostigmine Salicylate Dosages

Group	Dosage Level (mg/kg)
Phase I	
1	1.41
2	1.78
3	2.24
Phase II	
4(a) Males	2.35
4(b) Females	1.12
5(a) Males	1.59
5(b) Females	1.26
6 (vehicle control)	-

Chemical Analysis of Dosing Solution

The concentration of physostigmine salicylate in the dosing solutions was determined by UV spectrophotometry (Appendix A). Actual concentrations of physostigmine salicylate in the dosing solutions ranged from 96.1% to 99.2% of the target concentration.

Test Procedures

This study was conducted in accordance with LAIR SOP OP-STX-59 (5).

The volume of dosing solution each animal received was based upon the desired dose level, the compound's concentration in suspension, and the animal's weight. The dose volume was increased by varying the concentration of each suspension. Volumes ranged from 0.49 ml to 0.70 ml in the males and from 0.43 ml to 0.56 ml in females. The vehicle control group was given 0.46 ml to 0.66 ml sterile

water. The volumes given were based on 2.5 ml per kg. Dosing was performed without animal sedation or anesthesia. Sterile disposable 1-ml syringes (Becton, Dickerson & Co, Rutherford, NJ) fitted with 23-gauge, 3/4-inch needles were utilized. Animals in groups 1-3 were dosed between 0900 and 0947 hours on 3 June 1987, and animals in groups 4 and 5 were dosed between 0951 and 1020 hours on 4 June 1987.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (a) animals were observed undisturbed in their cages; (b) animals were removed from their cages and given a physical examination; and (c) animals were observed after being returned to their cages. On the day of the dosing, the animals were checked intermittently throughout the day. Recorded observations were performed 1, 2, and 4 hours after dosing, and daily for the remainder of the 2 week test period. A second "walk-through" observation was performed daily and only significant observations recorded. Body weights were recorded once weekly during the course of the study.

Necropsy

Animals that died during the observation period were submitted for a complete gross necropsy. Those which survived the 14-day study period were submitted for necropsy immediately after sacrifice by barbiturate overdose.

Statistical Analysis

Statistical analyses were performed on the study results. The LD10, LD50, and LD90 were derived by the maximum likelihood method of probit analysis, as described by Finney (6). The program, PROBIT, developed for the Data General Computer, Model MV8000, was used to determine the probit curve and lethal dose values.

Duration of Study

Appendix C is a complete listing of historical events.

Changes/Deviations

The dosing phase of this study was accomplished according to the protocol and applicable amendments with the following exceptions: The dosage levels for groups 4 and 5 were differentiated between males and females to allow a more

accurate MLD determination for each sex, the animals were identified by tail tattoo rather than eartag, and examination of the diaphragm by light microscopy was included as part of the necropsy findings. It is believed that none of these changes had any adverse effect on the results of this study.

Storage of Raw Data and Final Report

A copy of the final report, study protocol, raw data, retired SOPs and an aliquot of the test compound will be retained in the Letterman Army Institute of Research Archives.

RESULTS

Mortality

Fifty animals died as a result of the dosing. Forty-nine (98%) deaths occurred within 1 hour of dosing. One additional (2%) death occurred 7 days after dosing. Table 2 lists the compound-related deaths by group and the percent mortality. Appendix D is a tabular presentation of cumulative mortality.

Lethal Dose Calculations

Lethal dose values were calculated by probit analysis, and the equation for the probit regression line was: $Y = 1.77 + 12.90 \log X$ (males) and $Y = 3.08 + 10.24 \log X$ (females), where X is the dose and Y the corresponding probit value. Lethal doses calculated from the equation for the probit regression line are presented in Table 3. Figures 1 and 2 graphically present the actual data points and the regression line.

Clinical Observations

Forty-nine out of 110 dosed animals died within one hour of dosing and before any clinical signs were observed. Many of the remaining 61 dosed animals exhibited clinical signs within the first 24 hours after dosing. The clinical observations included behavioral, respiratory, reflexive, ocular, and gastrointestinal signs.

The most frequently observed category of signs was behavorial disturbances (50 of 61 animals). Behavioral signs exhibited by the animals included tremors, twitching, increased startle reflex, ataxia, and hypertonia. The one animal that died 7 days after dosing exhibited severe

TABLE 2: Compound-Related Deaths by Group

Group	Dose Level (mg/kg)	Compound Related Death/ Number in Group	Percent Mortality
		MALES	
1	1.41	1/10*	10
2	1.78	6/10	60
3	2.24	8/10	80
4(a)	2.35	10/10	100
5(a)	1.59	2/10	20
6	Control	0/ 5	0
		FEMALES	
1	1.41	5/10	50
2	1.78	6/10	60
3	2.24	10/10	100
4(b)	1.12	1/10	10
5(b)	1.26	1/10	10
6	Control	0/ 5	0

behavioral signs. Behavioral signs were present in all dose groups but the incidence decreased as the dose increased. This was due to the rapid onset of lethality precluding observation of a complete spectrum of clinical signs. Other classes of clinical signs observed and their frequencies included respiratory (28/61), gastrointestinal (18/61), reflexive (15/61), and ocular (8/61).

With the exception of the one animal in group 5(a) that died after 7 days, most clinical signs had cleared by 24 hours after dosing. The most common late term signs were irritability that developed after 5 or more days (30/61), staining near the ear tag or neck (9/61), nasal discharge/stain (19/61), and increase in startle reflex

TABLE 3: Calculated Lethal Doses (LD) of Physostigmine Salicylate in Sprague-Dawley Rats

Level	Calculated Dose[a] (mg/kg)	95% Confidence Limits (mg(base)/kg)
	MALES	
LD10	1.42 + 0.09	1.15 - 1.56
LD50	1.78 + 0.07	1.63 - 1.94
LD90	2.24 + 0.14	2.03 - 2.75
	FEMALES	
LD10	1.15 + 0.09	.90 - 1.29
LD50	1.54 + 0.08	1.39 - 1.73
LD90	2.05 + 0.19	1.80 - 2.78

[a] Calculated dose + standard error

(14/61). Tables 4 and 5 contain a summary of clinical observations. Appendix E contains individual animal histories.

Weight gains of survivors were not significantly affected by dosing. Table 6 presents the mean body weights by groups. Appendix F contains individual weight tables.

Pathology Findings

No gross lesions were observed in animals terminated at the end of the 14-day observation period. The observation of ocular or serous oronasal discharge in animals that died acutely is consistent with increased parasympathomimetic activity associated with physostigmine administration. Other findings were consistent with a convulsive terminal event. Non-specific myodegenerative changes were observed upon microscopic examination of the diaphragm of animals dying acutely. The pathologist's report is presented in Appendix G.

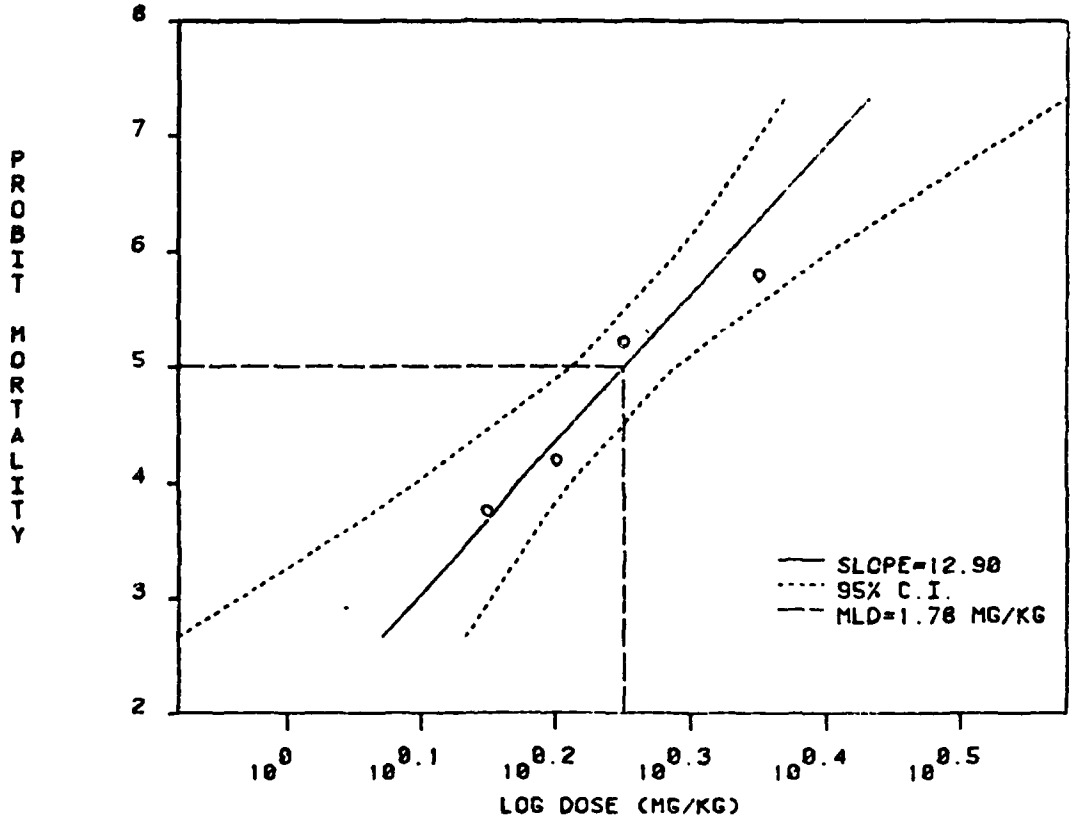


Figure 1: Physostigmine Dose Response Curve in Male Rats

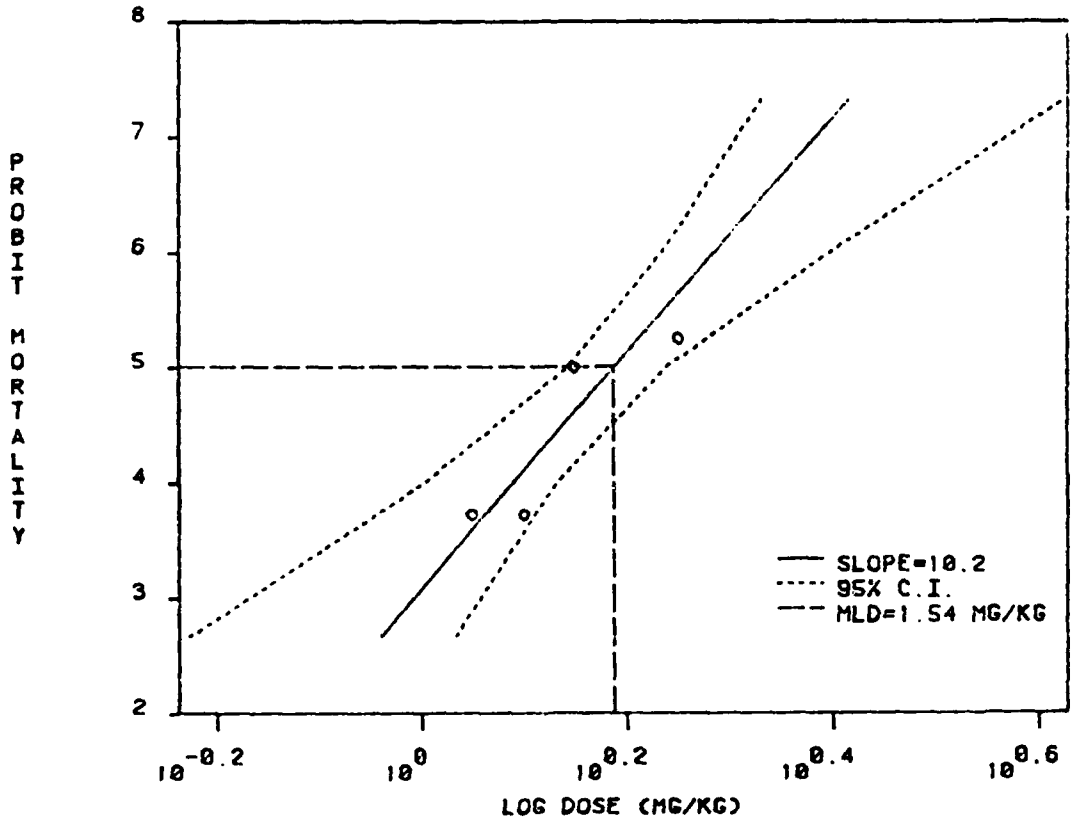


Figure 2: Physostigmine Dose Response Curve in Female Rats

TABLE 4: Incidence Summary for Clinical Observations in Male Rats Administered Physostigmine Salicylate

	Group	1	5(a)	2	3	4(a)	6
	Dose (mg/kg)	1.41	1.59	1.78	2.24	2.35	Vehicle Control
Clinical Signs	(n=)	10	10	10	10	10	5
General[a]		-	1	-	1	-	-
Behavior[b]		7	9	2	2	-	3
Reflexes[c]		-	1	-	-	-	-
Respiratory[d]		4	5	3	1	-	1
Ocular[e]		1	2	2	2	-	-
Gastrointestinal[f]		1	9	3	-	-	-
Death Before Signs Recorded		1	1	6	8	10	-

[a] Includes hunched posture.
[b] Includes inactive, hyperactive, irritable, ataxia, hypertonia, somnolence, twitching, and tremors.
[c] Includes hyperreflexia (startle).
[d] Includes gasping, nasal discharge, and staining.
[e] Includes lacrimation, chromodacryorrhea, and exophthalmos.
[f] Includes increased salivation and diarrhea.

TABLE 5: Incidence Summary for Clinical Observations in Female Rats Administered Physostigmine Salicylate

	Group	4(b)	5(b)	1	2	3	6
	Dose (*mg/kg*)	1.12	1.26	1.41	1.78	2.24	Vehicle Control
Clinical Signs	(n=)	10	10	10	10	10	5
Behavior[a]		9	9	3	3	-	3
Reflexes[b]		5	6	-	-	-	2
Respiratory[c]		1	1	1	2	-	1
Ocular[d]		-	-	-	1	-	-
Gastrointestinal[e]		1	3	1	-	-	-
Death Before Signs Recorded		1	1	5	6	10	-

a Includes inactive, hyperactive, irritable, ataxia, hypertonia, somnolence, and tremors.
b Includes hyperreflexia (startle).
c Includes gasping, nasal discharge, and staining.
d Includes lacrimation, chromodacryorrhea, and exophthalmos.
e Includes increased salivation and diarrhea.

TABLE 6: Mean Body Weights of Rats Administered Physostigmine Salicylate

Dose Groups (mg/kg)	At Receipt	At Dosing	Day 7	Termination Day 14[a]
		MALES		
1.41	136.9[b] ±2.6(10)	238.8 ±4.6(10)	282.3 ±3.6(9)	285.8 ±4.3(9)
1.59	138.4 ±2.1(10)	241.6 ±3.5(10)	255.9 ±13.7(9)	277.9 ±8.7(8)
1.78	139.7 ±3.6(10)	244.5 ±3.8(10)	272.5 ±11.6(4)	273.0 ±11.4(4)
2.24	137.7 ±3.6(10)	236.3 ±5.1(10)	281.0 ±8.0(2)	282.0 ±15.0(2)
2.35	137.9 ±2.9(10)	245.8 ±7.3(10)	-	-
Vehicle Control	138.6 ±1.2(5)	255.0 ±3.8(5)	297.2 ±5.4(5)	308.6 ±6.9(5)
		FEMALES		
1.12	156.3 ±1.3(10)	200.7 ±3.4(10)	214.0 ±2.1(9)	211.7 ±2.2(9)
1.26	158.4 ±1.8(10)	202.2 ±3.2(10)	208.1 ±7.6(9)	211.7 ±4.1(9)
1.41	155.4 ±1.8(10)	201.7 ±3.2(10)	209.2 ±3.7(5)	202.4 ±3.2(5)
1.78	155.9 ±3.4(10)	196.6 ±4.9(10)	217.8 ±6.5(4)	217.5 ±9.2(4)
2.24	155.9 ±2.6(10)	200.0 ±3.3(10)	-	-
Vehicle Control	156.8 ±3.2(5)	199.8 ±5.8(5)	211.6 ±5.5(5)	207.4 ±7.5(5)

[a]Weight after overnight fast.
[b]Values are mean±standard error (number of animals) in grams.

DISCUSSION

The acute subcutaneous administration of physostigmine salicylate to Sprague-Dawley rats produced pronounced toxicological effects. The calculated median lethal dose (MLD) for physostigmine salicylate in this study was 1.78 mg/kg in male and 1.54 mg/kg in female rats. This is consistent with previously reported MLD values for physostigmine which ranged from 1.28 to 1.67 mg/kg in male rats following intramuscular administration (7-9). These MLD values place physostigmine in the highly toxic classification (1-50 mg/kg) of Hodge and Sterner (10).

The toxicity observed following physostigmine administration was consistent with massive cholinergic stimulation following cholinesterase inhibition (11). Toxic signs attributable to excessive muscarinic stimulation included lacrimation, salivation, and diarrhea. The nicotinic effects observed included tremors, hypertonia, and irritability plus somnolence, inactivity, and ataxia as the animals became fatigued. These effects were observed primarily in surviving animals since those animals that received the higher doses died rapidly in convulsions without exhibiting the spectrum of toxic signs observed in animals receiving the lower doses.

The prolonged toxicity and late death observed in one male rat (87D00183) administered 1.59 mg/kg physostigmine salicylate are interesting. Since the half-life of physostigmine is extremely short, approximately 15 minutes following intravenous administration to rats (12), the mechanism for the delayed toxicity is not clear. Scheduled subchronic studies would be expected to clarify whether this was an idiosyncratic or a more generalized toxicological response.

CONCLUSION

Physostigmine salicylate is a highly toxic compound that produces clinical signs associated with cholinergic stimulation. Calculated MLD values were 1.78 mg/kg in male and 1.54 mg/kg in female Sprague-Dawley rats.

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APPENDICES

APPENDIX A: Chemical Data

Chemical Name: Physostigmine salicylate

Other Names: Eserine salicylate; Physostigmine, 2-hydroxybenzoate; 1, 2, 3, 3a, 8, 8a-Hexahydro-1, 3a, 8-trimethylpyrrolo[2,3-b]indol-5-ol methylcarbamate (ester), (3aS-cis)-, mono (2-hydroxybenzoate) (salt)

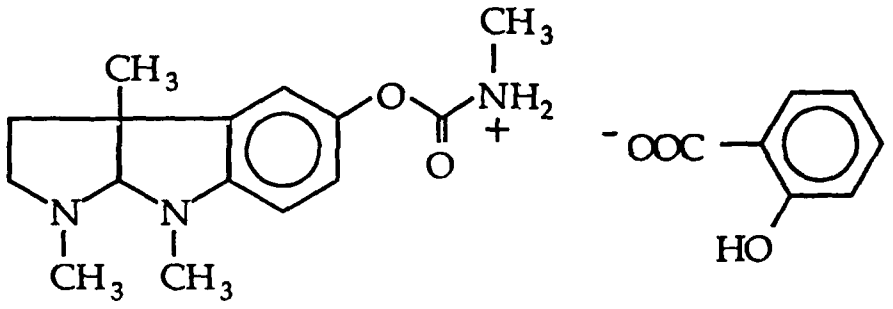
Lot Number: BL25591

Chemical Abstracts Service Registry Number: 57-64-7

LAIR Code: TW73

WRAIR Code: WR 6570AM

Chemical Structure:



Molecular Formula: $C_{15}H_{21}N_3O_2 \cdot C_7H_6O_3$

Molecular Weight: 413.47

Analytical Data:

The test compound was analyzed by the sponsors and the identity confirmed by UV and IR spectroscopy, high pressure liquid chromatography, mass spectrometry and elemental analysis.[1] Based on HPLC analysis of this test compound in comparison with the USP physostigmine salicylate reference standard, lot BL25591 contains 66.7% (100.1% of theory) physostigmine base and 33.7% (100.8% of theory) salicylic acid or 100.4% physostigmine salicylate.[1]

HPLC analysis of physostigmine salicylate in this lab was performed using a Hewlett-Packard 1090 HPLC system equipped with a diode array detector. The compound was chromatographed under the following conditions: silica

APPENDIX A (cont.): Chemical Data

column (4.6 x 100 mm, Brownlee Labs, Inc.); mobile phase, 15% acetonitrile/buffer (0.01M Na_2HPO_4 with 0.0025M tetramethylammonium chloride); flow rate, 1.5 ml/min; wavelength monitored, 210 nm. The compound eluted as two peaks with retention times of 0.9 min (salicylic acid), and 3.9 min (physostigmine).[2]

IR (KBr): 3320(broad), 2964, 2325, 1744, 1629, 1594, 1485, 1460, 1383, 1326, 1245, 1203, 1184, 1151, 1140, 1087, 1006, 993, 944, 860, 807, 754, 704, 667, 382 cm^{-1}.[3] The IR spectrum was identical to that provided by the sponsors[1].

Source: Bill Ellis
Division of Experimental Therapeutics
Walter Reed Army Institute of Research
Washington, DC
Requested by LTC Jurgen von Bredow, PhD, MSC

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[1]Masamori E, Benitez A, and Lim P. Assay of physostigmine salicylate, WR-6570AM, BL25591. Menlo Park, CA: SRI International, 4 November 1986; Report no. 553.

[2]Wheeler CR. Toxicity testing of antidotes of chemical warfare agents. Laboratory notebook #85-12-024.1, pp 2-11. Letterman Army Institute of Research, Presidio of San Francisco, CA.

[3]Wheeler CR. Toxicity testing of antidotes of chemical warfare agents. Laboratory notebook #85-12-024.3, pp 10-11. Letterman Army Institute of Research, Presidio of San Francisco, CA.

APPENDIX A (cont.): Chemical Data

ANALYSIS OF PHYSOSTIGMINE SALICYLATE DOSING SOLUTIONS

The concentration of physostigmine salicylate in dosing solutions was deterr ned by UV absorbance at 298 nm. Each solution was diluted and the absorbance measured by a Hitachi 110A Spectrophotometer. Using an extinction coefficient of 6222 L/moles·cm the concentration of physostigmine salicylate was calculated. The concentrations of dosing solutions for GLP study number 87005 are presented below:

Date of Sample Preparation & Analysis	Concentration (mg/ml) Target	Actual	% Target
3 June 87*	0.564	0.542	96.1
	0.712	0.684	96.1
	0.896	0.864	96.4
4 June 87†	0.448	0.444	99.1
	0.504	0.495	98.2
	0.636	0.631	99.2
	0.940	0.927	98.6

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*Wheeler CR. Toxicity testing of antidotes for chemical warfare agents. Laboratory Notebook #85-12-024, p 41. Letterman Army Institute of Research, Presidio of San Francisco, CA.

†*Ibid*, p 42.

APPENDIX B: Animal Data

Species: *Rattus norvegicus*

Strain: Sprague-Dawley

Source: Bantin-Kingman, Inc
Fremont, CA

Sex: Male and female

Date of birth: Male: 7 April 1987
Female: 6 April 1987

Method of randomization: Weight bias, stratified animal allocation (Beckman TOXSYS® Animal Allocation Program, SOP OP-1SG-24)

Animals in each group: 10 male and 10 female animals - Group 1-5 - 5 each for vehicle control groups

Condition of animals at start of study: Normal

Body weight range at dosing: 172 - 281 g

Identification procedures: Tail tattoo.

Pretest conditioning: Quarantine/acclimation 21 May - 2 June 1987

Justification: The laboratory rat has proven to be a sensitive and reliable animal model for lethal dose determinations.

APPENDIX C: Historical Listing of Study Events

Date	Event
21 May 87	Rats arrived and were checked for physical condition, sexed, weighed, and individually caged.
22 May 87	Four rats (2 male and 2 female) were submitted for necropsy quality control. Animals were tattooed on the tail.
22 May - 3 Jun 87	Animals were observed daily.
1 Jun 87	Animals were weighed and randomized into dose groups.
3 Jun 87	Phase I animals (Groups 1-3) were weighed, dosed, and observed at 1, 2, and 4 hours after dosing.
3-16 Jun 87	Phase I animals were observed daily in a.m. and p.m.
4 Jun 87	Phase II animals (Groups 4-6) were weighed, dosed, and observed at 1, 2 and 4 hours after dosing.
4-17 Jun 87	Phase II animals were observed daily in a.m. and p.m.
10 Jun 87	Phase I animals were weighed.
11 Jun 87	Phase II animals were weighed.
16 Jun 87	Food was removed in p.m. from all Phase I animals.
17 Jun 87	All surviving Phase I animals were observed, weighed, sacrificed, and submitted for necropsy.
17 Jun 87	Food was removed in p.m. from all Phase II animals.
18 Jun 87	All surviving Phase II animals were observed, weighed, sacrificed, and submitted for necropsy.

APPENDIX D: Cumulative Mortality Data (Death/Group)
(10 Animals Per Group)

Time After Dosing												
Dose	Hours				Days							
mg/kg	1	2	4	12	1	2	3	4	5	6	7	8-14
Males												
1.41	1	1	1	1	1	1	1	1	1	1	1	1
1.59	1	1	1	1	1	1	1	1	1	1	2	2
1.78	6	6	5	6	6	6	6	6	6	6	6	6
2.24	8	8	8	8	8	8	8	8	8	8	8	8
2.35	10	10	10	10	10	10	10	10	10	10	10	10
0[a]	0	0	0	0	0	0	0	0	0	0	0	0
Females												
1.12	1	1	1	1	1	1	1	1	1	1	1	1
1.26	1	1	1	1	1	1	1	1	1	1	1	1
1.41	5	5	5	5	5	5	5	5	5	5	5	5
1.78	6	6	6	6	6	6	6	6	6	6	6	6
2.24	10	10	10	10	10	10	10	10	10	10	10	10
0[a]	0	0	0	0	0	0	0	0	0	0	0	0
TOTAL	49	49	49	49	49	49	49	49	49	49	50	50

[a] 5 animals per group

APPENDIX E: INDIVIDUAL ANIMAL HISTORIES

MALE: 1.41 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00136	Somnolence	June 3	Moderate
	Nasal Discharge(red-brown)	June 3	Moderate
	Ataxia	June 3	Slight
	Inactive	June 6,7	Slight
87D00144	Inactive	June 3	Slight
	Nasal Discharge(red-brown)	June 3	Moderate
	Exophthalmos	June 3	Present
	Diarrhea	June 5	Present
	Inactive	June 7,8	Slight
	Irritable	June 11,12	Moderate
87D00158	Normal	N/A	N/A
87D00163	Inactive	June 6	Slight
87D00165	Material, Nose, Red, Dark	June 6,8	Slight
	Irritable	June 8,12,15,16	Moderate
87D00177	Normal	N/A	N/A
87D00186	Death	June 3	0.6 h
87D00190	Inactive	June 6,7	Moderate
87D00193	Inactive	June 6	Slight
	Irritable	June 8,11,12	Moderate
87D00194	Inactive	June 6	Slight
	Material Dark, Nose	June 8	Slight
	Stain Dark, Red, Nose	June 13,14	Slight
	Irritable	June 8	Moderate

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1.78 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00146	Lacrimation	June 3	Slight
	Nasal Discharge(red-brown)	June 3	Slight
	Inactive	June 3	Slight
87D00147	Death	June 3	0.5 h
87D00157	Inc. Salivation(lt. brown)	June 3	Slight
	Nasal Discharge(red-brown)	June 3	Slight
	Material Dark Nose	June 8-12,15	Slight
87D00161	Death	June 3	0.8 h
87D00167	Death	June 3	0.3 h
87D00175	Death	June 3	0.5 h
87D00178	Chromodacyorrhea	June 3	Moderate
	Inc. Salivation(lt. brown)	June 3	Slight
	Nasal Discharge(red-brown)	June 3	Moderate
87D00188	Death	June 3	0.5 h
87D00192	Inc. Salivation(lt. brown)	June 3	Present
	Inactive	June 6,7	Slight
87D00199	Death	June 3	0.5 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 2.24 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00135	Death	June 3	0.4 h
87D00148	Ataxia	June 3	Slight
	Chromodacryorrhea	June 3	Slight
	Exophthalmos	June 3	Present
	Nasal Discharge(red-brown)	June 3	Slight
	Hunched Posture	June 6	Slight
	Material Dark Nose	June 8	Slight
	Irritable	June 9-12,15,16	Moderate
87D00151	Death	June 3	0.5 h
87D00156	Death	June 3	0.5 h
87D00159	Death	June 3	0.4 h
87D00181	Death	June 3	0.3 h
87D00182	Death	June 3	0.3 h
87D00184	Death	June 3	0.3 h
87D00191	Death	June 3	0.3 h
87D00197	Inactive	June 3	Moderate
	Chromodacryorrhea	June 3	Slight
	Exophthalmos	June 3	Present
	Tremors	June 3	Moderate
	Irritable	June 9	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 2.35 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00137	Death	June 4	0.2 h
87D00142	Death	June 4	0.3 h
87D00150	Death	June 4	0.4 h
87D00152	Death	June 4	0.3 h
87D00160	Death	June 4	0.4 h
87D00170	Death	June 4	0.4 h
87D00173	Death	June 4	0.4 h
87D00176	Death	June 4	0.4 h
87D00189	Death	June 4	0.3 h
87D00195	Death	June 4	0.6 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1.59 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00143	Ataxia	June 4	Moderate
	Inc. Salivation	June 3	Marked
	Inactive	June 6	Slight
	Irritable	June 15-17	Moderate
87D00169	Tremors	June 4	Slight
	Ataxia	June 4	Moderate
	Inc. Salivation	June 4	Moderate
	Irritable	June 4	Slight
87D00171	Inc. Salivation	June 4	Moderate
	Chromodacryorrhea	June 4	Slight
	Ataxia	June 4	Slight
	Tremors	June 4	Slight
87D00172	Hypertonia	June 4	Moderate
	Ataxia	June 4	Moderate
	Tremors	June 4	Slight
	Inc. Salivation	June 4	Moderate
	Irritable	June 5,10,11	Slight
	Stain Dark Red, Nose	June 14	Slight
87D00174	Inactive	June 4,5	Moderate
	Tremors	June 4	Slight
	Ataxia	June 4	Moderate
	Hypertonia	June 4	Slight
	Chromodacryorrhea	June 4	Slight
	Inc. Salivation	June 4	Moderate
	Nasal Discharge(red-brown)	June 4	Slight
	Irritable	June 8	Slight
	Material Red, Nose	June 9	Slight
87D00180	Tremors	June 4	Slight
	Ataxia	June 4	Slight
	Inc. Salivation	June 4	Slight
	Irritable	June 8	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: 1.59 mg/kg Physostigmine salicylate (cont.)

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00183	Ataxia	June 4	Marked
	Twitching	June 4	Marked
	Inc. Salivation	June 4	Marked
	Hypertonia	June 4-6	Marked
	Material Red, Head	June 7,8	Marked
	Inc. Startle Reflex	June 8-10	Marked
	Material Red, Body	June 9,10	Marked
	Hunched Posture	June 10	Present
	Death	June 11	7 days
87D00185	Death	June 4	0.5 h
87D00187	Inc. Salivation	June 4	Moderate
	Inactive	June 4,6	Moderate
	Ataxia	June 4	Slight
	Tremors	June 4	Slight
87D00200	Tremors	June 4	Slight
	Ataxia	June 4	Moderate
	Gasping	June 4	Slight
	Inc. Salivation	June 4	Marked
	Nasal Discharge(re-brown)	June 4	Moderate
	Irritable	June 9,11	Moderate

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

MALE: Vehicle Control

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00145	Irritable	June 11,12,16,17	Slight
87D00153	Material Red, Nose	June 10	Slight
87D00162	Irritable	June 4,8,9	Slight
87D00179	Irritable	June 4,12,13	Slight
87D00201	Normal	N/A	N/A

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1.41 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00215	Nasal Discharge(red-brown)	June 3	Slight
87D00216	Irritable	June 11	Slight
87D00218	Death	June 3	0.6 h
87D00219	Death	June 3	0.4 h
87D00222	Stain Red, Ears	June 14,15	Slight
87D00225	Death	June 3	0.5 h
87D00233	Inc. Salivation(lt. brown)	June 3	Slight
	Ataxia	June 3	Moderate
	Inactive	June 3	Moderate
	Irritable	June 8-12,15,16	Moderate
87D00236	Death	June 3	0.9 h
87D00240	Inactive	June 3	Slight
87D00247	Death	June 3	0.8 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1.78 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00226	Nasal Discharge(red-brown)	June 3	Slight
	Inactive	June 3	Slight
	Irritable	June 8-12,15,16	Moderate
87D00230	Stain Red, Ear	June 14,15	Slight
87D00234	Death	June 3	0.7 h
87D00248	Death	June 3	0.7 h
87D00250	Death	June 3	0.9 h
87D00252	Inactive	June 3	Slight
	Lacrimation	June 3	Slight
	Irritable	June 9,10,16	Marked
87D00253	Nasal Discharge(red-brown)	June 3	Slight
	Inactive	June 3	Slight
	Irritable	June 7	Slight
	Stain Red, Head	June 8-11	Slight
	Stain Red, Neck	June 12-17	Moderate
	Stain Red, Nose	June 12-17	Slight
	Stain Red, Ear	June 14-17	Slight
87D00258	Death	June 3	0.8 h
87D00259	Death	June 3	0.7 h
87D00269	Death	June 3	0.6 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 2.24 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00202	Death	June 3	0.5 h
87D00204	Death	June 3	0.4 h
87D00205	Death	June 3	0.4 h
87D00206	Death	June 3	0.6 h
87D00207	Death	June 3	0.4 h
87D00213	Death	June 3	0.4 h
87D00249	Death	June 3	0.2 h
87D00264	Death	June 3	0.3 h
87D00266	Death	June 3	0.4 h
87D00268	Death	June 3	0.3 h

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1.12 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00208	Death	June 4	0.6 h
87D00212	Tremors	June 4	Slight
	Ataxia	June 4	Slight
	Inc. Startle Reflex	June 8-12,15 17,18	Slight
	Irritable	June 15	Moderate
87D00214	Tremors	June 4	Slight
	Ataxia	June 4	Slight
	Inc. Startle Refles	June 15,17,18	Slight
	Stain Red, Ears	June 16-18	Slight
87D00223	Tremors	June 4	Slight
	Hypertonia	June 4	Moderate
	Inc. Startle Refles	June 15	Slight
	Irritable	June 15-17	Moderate
87D00239	Tremors	June 4	Slight
	Ataxia	June 4	Moderate
	Irritable	June 15	Moderate
	Material Red, Nose	June 16	Slight
87D00242	Tremors	June 4	Moderate
	Inactive	June 4	Slight
	Irritable	June 9-11,15	Marked
87D00243	Tremors	June 4	Moderate
	Inactive	June 4	Moderate
	Inc. Startle Reflex	June 15	Slight
87D00256	Tremors	June 4	Moderate
	Ataxia	June 4	Slight
	Inc. Startle Reflex	June 8-10,12	Slight
		15-18	Moderate
	Irritable	June 11	Slight
	Hyperactive	June 16-18	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1.12 mg/kg Physostigmine salicylate (cont.)

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00260	Tremors	June 4	Slight
	Hypertonia	June 4	Slight
	Ataxia	June 4	Slight
	Inc. Salivation(clear)	June 4	Moderate
	Irritable	June 15	Moderate
87D00263	Tremors	June 4	Slight
	Ataxia	June 4	Slight
	Irritable	June 9,11,12	Slight
		15-17	Moderate

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1.26 mg/kg Physostigmine salicylate

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00211	Tremors	June 4	Moderate
	Ataxia	June 4	Moderate
	Inactive	June 6,7	Moderate
	Inc. Startle Reflex	June 11	Slight
87D00221	Tremors	June 4	Slight
	Ataxia	June 4	Moderate
	Hypertonia	June 4	Moderate
	Irritable	June 4,9,15	Moderate
	Inc. Startle Reflex	June 15-17	Slight
87D00231	Tremors	June 4	Slight
	Ataxia	June 4	Moderate
	Stain Red, Ear	June 12-18	Moderate
	Inc. Startle Reflex	June 15-18	Moderate
	Stain Red, Neck	June 15-18	Slight
87D00238	Death	June 4	0.4 h
87D00241	Tremors	June 4	Moderate
	Ataxia	June 4	Moderate
	Inactive	June 7,8	Slight
	Inc. Startle Reflex	June 10-12	Slight
	Stain Red, Neck	June 11	Slight
	Stain Dark Red, Ear	June 14,15	Slight
87D00244	Tremors	June 4	Moderate
	Hypertonia	June 4	Moderate
	Inc. Salivation	June 4	Moderate
	Nasal Discharge(red-brown)	June 4	Slight
	Stain Red, Head	June 8,9	Slight
	Irritable	June 11,15	Slight
	Stain Red, Neck	June 11-14	Slight
	Stain Red, Ears	June 14-18	Slight
	Material Red, Nose	June 16-18	Slight
87D00257	Tremors	June 4	Slight
	Ataxia	June 4	Slight

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: 1.26 mg/kg Physostigmine salicylate (cont.)

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00261	Tremors	June 4	Moderate
	Ataxia	June 4	Slight
	Inc. Startle Reflex	June 10,11	Slight
87D00265	Tremors	June 4	Slight
	Ataxia	June 4	Moderate
	Hypertonia	June 4	Marked
	Inc. Salivation	June 4	Slight
	Inc. Startle Reflex	June 8-12	Slight
	Irritable	June 8,9,11 15-17	Moderate
87D00267	Tremors	June 4	Slight
	Ataxia	June 4	Moderate
	Hypertonia	June 4	Marked
	Inc. Salivation	June 4	Moderate
	Stain Red, Neck	June 11-13,15	Slight
	Irritable	June 12, 15-17	Moderate

APPENDIX E (cont.): INDIVIDUAL ANIMAL HISTORIES

FEMALE: Vehicle Controls

Animal Number	Clinical Signs	Dates Observed (1987)	Severity
87D00203	Irritable	June 11,12, 15-17	Moderate
87D00210	Stain Red, Ear	June 16-18	Slight
87D00220	Material Red, Nose	June 9	Slight
87D00232	Inc. Startle Reflex	June 8-12, 17,18	Moderate
	Hyperactive	June 16	Slight
	Irritable	June 17,18	Moderate
87D00251	Irritable	June 9,11, 14-16	Moderate
	Inc. Startle Reflex	June 10	Moderate
	Stain Red, Ears	June 12-17	Moderate

APPENDIX F: Individual Body Weights

MALES 1.41 mg/kg - Group 1

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14[a]
87D00136	129[b]	228	280	289
87D00144	127	214	271	275
87D00158	144	225	281	287
87D00163	149	246	292	299
87D00165	143	255	278	279
87D00177	148	264	305	309
87D00186	131	235	Dead	-
87D00190	130	241	271	270
87D00193	135	236	276	273
87D00194	133	244	287	291
Mean	136.9	238.8	282.3	285.8
Standard Deviation	8.3	14.6	10.9	12.9
Std. Error of Mean	2.6	4.6	3.6	4.3

[a] Weights after an overnight fast.
[b] Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 1.78 mg/kg - Group 2

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14[a]
87D00146	143[b]	233	253	257
87D00147	120	232	Dead	-
87D00157	151	258	299	301
87D00161	140	246	Dead	-
87D00167	137	239	Dead	-
87D00175	144	260	Dead	-
87D00178	138	231	253	252
87D00188	145	260	Dead	-
87D00192	144	251	285	282
87D00199	135	235	Dead	-
Mean	139.7	244.5	272.5	273.0
Standard Deviation	8.3	12.0	23.2	22.8
Std. Error of Mean	2.6	3.8	11.6	11.4

[a] Weights after an overnight fast.
[b] Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 2.24 mg/kg - Group 3

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14[a]
87D00135	151[b]	246	Dead	-
87D00148	139	240	289	297
87D00151	139	244	Dead	-
87D00156	141	246	Dead	-
87D00159	138	248	Dead	-
87D00181	115	210	Dead	-
87D00182	121	207	Dead	-
87D00184	143	225	Dead	-
87D00191	142	250	Dead	-
87D00197	148	247	273	267
Mean	137.7	236.3	281.0	282.0
Standard Deviation	11.2	16.3	11.3	21.2
Std. Error of Mean	3.6	5.1	8.0	15.0

[a] Weights after an overnight fast.
[b] Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 2.35 mg/kg - Group 4(a)

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14[a]
87D00137	125[b]	232	Dead	-
87D00142	126	196	Dead	-
87D00150	134	255	Dead	-
87D00152	128	229	Dead	-
87D00160	143	281	Dead	-
87D00170	149	241	Dead	-
87D00173	140	250	Dead	-
87D00176	141	255	Dead	-
87D00189	143	260	Dead	-
87D00195	150	259	Dead	-
Mean	137.9	245.8	-	-
Standard Deviation	9.2	23.0	-	-
Std. Error of Mean	2.9	7.3	-	-

[a] Weights after an overnight fast.
[b] Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

MALES 1.59 mg/kg - Group 5(a)

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14[a]
87D00143	128[b]	246	285	301
87D00169	142	237	293	315
87D00171	147	240	272	281
87D00172	130	226	247	252
87D00174	137	259	281	297
87D00180	141	242	264	266
87D00183	149	261	155	Dead
87D00185	137	237	Dead	-
87D00187	138	234	257	264
87D00200	135	234	249	247
Mean	138.4	241.6	255.9	277.9
Standard Deviation	6.7	11.1	41.1	24.6
Std. Error of Mean	2.1	3.5	13.7	8.7

[a] Weights after an overnight fast.
[b] Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 1.41 mg/kg - Group 1

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14[a]
87D00215	149[b]	194	202	195
87D00216	151	195	203	199
87D00218	166	226	Dead	-
87D00219	156	197	Dead	-
87D00222	159	210	223	212
87D00225	152	199	Dead	-
87D00233	158	203	209	208
87D00236	155	204	Dead	-
87D00240	148	192	209	198
87D00247	160	197	Dead	-
Mean	155.4	201.7	209.2	202.4
Standard Deviation	5.6	10.1	8.4	7.2
Std. Error of Mean	1.8	3.2	3.7	3.2

[a] Weights after an overnight fast.
[b] Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 1.78 mg/kg - Group 2

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14[a]
87D00226	161[b]	209	220	233
87D00230	153	209	211	224
87D00234	157	191	Dead	-
87D00248	143	190	Dead	-
87D00250	157	201	Dead	-
87D00252	160	191	205	191
87D00253	171	215	235	222
87D00258	146	172	Dead	-
87D00259	140	174	Dead	-
87D00269	171	214	Dead	-
Mean	155.9	196.6	217.8	217.5
Standard Deviation	10.7	15.6	13.0	18.3
Std. Error of Mean	3.4	4.9	6.5	9.2

[a] Weights after an overnight fast.
[b] Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 2.24 mg/kg - Group 3

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14[a]
87D00202	159[b]	195	Dead	-
87D00204	166	214	Dead	-
87D00205	148	180	Dead	-
87D00206	164	205	Dead	-
87D00207	159	204	Dead	-
87D00213	151	94	Dead	-
87D00249	157	200	Dead	-
87D00264	163	216	Dead	-
87D00266	139	193	Dead	-
87D00268	153	199	Dead	-
Mean	155.9	200.0	-	-
Standard Deviation	8.3	10.6	-	-
Std. Error of Mean	2.6	3.3	-	-

[a] Weights after an overnight fast.
[b] Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 1.12 mg/kg - Group 4(b)

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14[a]
87D00208	157[b]	183	Dead	-
87D00212	155	207	221	221
87D00214	158	195	214	211
87D00223	160	183	206	218
87D00239	155	203	209	202
87D00242	159	210	216	206
87D00243	158	206	225	219
87D00256	149	208	208	210
87D00260	162	214	217	212
87D00263	150	198	210	206
Mean	156.3	200.7	214.0	211.7
Standard Deviation	4.2	10.8	6.4	6.5
Std. Error of Mean	1.3	3.4	2.1	2.2

[a] Weights after an overnight fast.
[b] Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

FEMALES 1.26 mg/kg - Group 5(b)

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14[a]
87D00211	166[b]	205	213	206
87D00221	152	190	195	203
87D00231	154	191	201	201
87D00238	155	199	Dead	-
87D00241	162	193	211	208
87D00244	151	205	218	222
87D00257	166	222	239	233
87D00261	161	198	208	198
87D00265	163	210	229	226
87D00267	154	209	159	208
Mean	158.4	202.2	208.1	211.7
Standard Deviation	5.8	10.0	22.8	12.3
Std. Error of Mean	1.8	3.2	7.6	4.1

[a] Weights after an overnight fast.
[b] Weights are given in grams.

APPENDIX F (cont.): Individual Body Weights

VEHICLE CONTROLS

Animal No.	At Receipt	At Dosing	Day 7	Termination Day 14[a]
		MALES		
87D00145	143[b]	254	282	288
87D00153	137	254	300	313
87D00162	139	263	300	306
87D00179	137	262	314	331
87D00201	137	242	290	305
Mean	138.6	255.0	297.2	308.6
Standard Deviation	2.6	8.4	12.0	15.5
Std. Error of Mean	1.2	3.8	5.4	6.9
		FEMALES		
87D00203	164	213	214	208
87D00210	165	200	225	213
87D00220	152	184	201	194
87D00232	154	212	221	232
87D00251	149	190	197	190
Mean	156.8	199.8	211.6	207.4
Standard Deviation	7.3	12.9	12.2	16.7
Std. Error of Mean	3.2	5.8	5.5	7.5

[a] Weights after an overnight fast.
[b] Weights are given in grams.

APPENDIX G: Pathology Report

Pathology Report GLP 87005

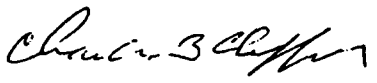
Acute Subcutaneous Toxicity of
Physostigmine Salicylate in Rats

I. Prinicpal Investigator: CPT Denzil F. Frost
Mammalian Toxicology
Pathologist: MAJ Charles B. Clifford

II. Rattus norvegicus, Sprague-Dawley, 125-175 grams.

III. Comment: Gross changes observed in animals which succumbed in the first 24 hours are considered either as evidence of increased parasympathetic activity (ocular and oronasal discharge) or as changes of a non-specific nature (pulmonary and utero-ovarian congestion) frequently observed in animals dying from a wide variety of causes. A role for physostigmine in the latter was considered unlikely. None of these gross changes were considered as evidence of tissue damage. Microscopic alterations observed in diaphragms from animals in the high dose group were acute non-specific myodegenerative changes seen in animals dying from a wide variety of causes.

No lesions were observed in rats necropsied 14 days (terminal sacrifice) after dosing with physostigmine salicylate.



CHARLES B. CLIFFORD, DVM
MAJ, VC
Division of Pathology

16 November 1987/dbj

APPENDIX G (cont.): Pathology Report

ATTACHMENT:

GROUP #1 (1.41 mg/kg) 20 rats, 14 sacrificed 17 Jun 87.

ANIMAL ID#	LAIR ACC#	SEX	DIAGNOSES
87D00144	41060	M	No lesions recognized
136	41061	M	No lesions recognized
158	41072	M	No lesions recognized
163	41073	M	No lesions recognized
165	41074	M	No lesions recognized
177	41075	M	No lesions recognized
190	41076	M	No lesions recognized
193	41077	M	No lesions recognized
194	41078	M	No lesions recognized
215	41079	F	No lesions recognized
222	41080	F	No lesions recognized
216	41081	F	No lesions recognized
233	41082	F	No lesions recognized
240	41083	F	No lesions recognized

GROUP #1 Spontaneous Deaths, 6 rats
Necropsy Date: 3 June 1987

87D00186	40937	M	Red-tinged ocular discharge. Serous oronasal discharge. Pulmonary congestion.
218	40944	F	Serous oronasal discharge. Pulmonary congestion.
219	40945	F	Serous oronasal discharge. Pulmonary congestion.
225	40946	F	Serous oronasal congestion, red-tinged ocular discharge. Utero-ovarian congestion.
236	40950	F	Serous oronasal discharge. Red-tinged ocular discharge.
244	40953	F	Serous oronasal discharge. Pulmonary congestion.

GROUP #2 (1.78 mg/kg) 20 rats, 8 rats sacrificed 17 Jun 87

87D00146	41071	M	No lesions recognized
157	41070	M	No lesions recognized
178	41069	M	No lesions recognized
192	41068	M	No lesions recognized
226	41067	F	No lesions recognized
230	41066	F	No lesions recognized
252	41065	F	No lesions recognized
253	41064	F	No lesions recognized

APPENDIX G (cont.): Pathology Report

GROUP #2 Spontaneous deaths, 12 rats
Necropsy Date: 3 June 1987

ANIMAL ID#	LAIR ACC#	SEX	DIAGNOSES
87D00188	40929	M	Serous oronasal discharge.
199	40930	M	Serous oronasal discharge.
147	40933	M	Serous oronasal discharge.
161	40961	M	Serous oronasal discharge.
167	40935	M	Red-tinged ocular discharge. Serous oronasal discharge. Pulmonary congestion.
175	40936	M	Serous oronasal discharge. Pulmonary congestion. Serous oronasal discharge.
234	70951	F	Pulmonary congestion. Serous oronasal discharge.
248	40955	F	Pulmonary congstion. Serous oronasal discharge.
250	40947	F	Serous oronasal discharge.
259	40948	F	Serous oronasal discharge. Utero-ovarian congestion.
258	70952	F	Serous oronasal discharge. Pulmonary congestion.
269	40956	F	Red-tinged ocular and serous oronasal discharge. Pulmonary congestion.

GROUP 3 (2.24 mg/kg) 20 rats, 2 rats secrificed 17 Jun 87

87D00148	41062	M	No lesions recognized.
197	41063	M	No lesions recognized.

GROUP 3 (Spontaneous Deaths) 18 rats
Necropsy date: 3 Jun 87

87D00135	40932	M	Serous oronasal discharge #1
151	40960	M	Serous oronasal discharge #1
156	40934	M	Serous cculonasal discharge #1
159	40931	M	Serous oral discharge #1
181	40925	M	Blood in oral cavity, Serous oral discharge, #1
182	40926	M	Serous oral discharge #1
184	40927	M	Red-tinged oral discharge, #1, #2
191	40928	M	Serous oral discharge, #1
202	40938	F	Serous oronasal discharge, #1
204	40939	F	Serous oronasal discharge #1
205	40940	F	Serous oronasal discharge #1
206	40941	F	Serous oronasal discharge, #1
207	40949	F	Serous oronasal discharge #1
213	40943	F	Serous oronasal discharge, #1
249	40954	F	Serous oronasal discharge, #1
264	40959	F	Oronasal discharge, #1

APPENDIX G (cont.): Pathology Report

GROUP 3 Spontaneous deaths (Continued)

ANIMAL ID#	LAIR ACC#	SEX	DIAGNOSES
266	40957	F	Serous oronasal discharge, #1
268	40958	F	Serous oronasal discharge, #1

#1 Microscopic examination of diaphragm - Mild, multifocal, swelling and hyalinization of some myofibers.

#2 Microscopic examination of diaphragm - Minimal, multifocal, hemorrhage.

GROUP 4A (1.12 mg/kg) 10 rats, all males, all died 4 Jun 87.

ANIMAL ID#	LAIR ACC#	DIAGNOSES
87D00137	70963	Red-tinged ocular and serous oronasal discharged. Pulmonary congestion.
142	40964	Serous oronasal discharge
150	40965	Red-tinged ocular and serous oronasal discharge. Pulmonary congestion.
152	40966	Red-tinged ocular and serous oronasal discharge
160	40967	Red-tinged ocular and serous oronasal discharge. Pulmonary congestion.
170	40968	Red-tinged ocular and serous oronasal discharge.
173	40969	Serous oronasal discharge.
176	40970	Serous oronasal discharge, Pulmonary congestion.
189	40971	Serous oronasal discharged.
195	40972	Red-tinged ocular and serous oronasal discharge. Pulmonary congestion.

GROUP 4B (1.12 mg/kg) 10 rats, 9 rats, sacrificed 18 Jun 87.

87D00212	41086	F	No lesions recognized.
214	41087	F	No lesions recognized.
223	41088	F	No lesions recognized.
239	41090	F	No lesions recognized.
242	41089	F	No lesions recognized.
243	41091	F	No lesions recognized.
256	41092	F	No lesions recognized.
260	41093	F	No lesions recognized.
263	41094	F	No lesions recognized.
Spontaneous Death			
87D00208	40974	F	Serous oral nasal discharge, pulmonary congestion.

APPENDIX G (cont.): Pathology Report

GROUP 5A (1.59 mg/kg) 10 rats, 8 rats sacrificed 18 Jun 87.

ANIMAL ID#	LAIR ACC#	SEX	DIAGNOSES
87D00143	41114	M	No lesions recognized.
169	41115	M	No lesions recognized.
171	41116	M	No lesions recognized.
172	41117	M	No lesions recognized.
174	41118	M	No lesions recognized.
180	41119	M	No lesions recognized.
187	41120	M	No lesions recognized.
200	41121	M	No lesions recognized.

GROUP 5A (Spontaneous deaths) 2 rats, both male.

ANIMAL ID#	LAIR ACC#	Necropsy DATE	DIAGNOSES
87D00183	41028	11 Jun 87	#3
185	40973	4 Jun 87	Serous oronasal discharge. Pulmonary congestion.

#3 The advanced state of autolysis precluded meaningful gross pathologic evaluation.

GROUP 5B (1.26 mg/kg) 10 rats, 9 rats sacrificed 18 Jun 87.

ANIMAL ID#	LAIR ACC#	SEX	DIAGNOSES
87D00211	41095	F	No lesions recognized.
221	41096	F	No lesions recognized.
231	41097	F	No lesions recognized.
241	41098	F	No lesions recognized.
244	41099	F	No lesions recognized.
257	41100	F	No lesions recognized.
261	41101	F	No lesions recognized.
265	41102	F	No lesions recognized.
267	41103	F	No lesions recognized.

GROUP 5B (Spontaneous deaths) 1 rat, 4 Jun 87.

87D00238	40975	F	No lesions recognized

GROUP 6 (Control - 0 mg/kg) 10 rats, all sacrificed 18 Jun 87.

87D00203	41104	F	No lesions recognized.
210	41105	F	No lesions recognized.
220	41106	F	No lesions recognized.
232	41107	F	No lesions recognized.
251	41108	F	No lesions recognized.
145	41109	M	No lesions recognized.
153	41110	M	No lesions recognized.
162	41111	M	No lesions recognized.
179	41112	M	No lesions recognized.
201	41113	M	No lesions recognized.

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